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Original Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF ATAZANVIR SULPHATE AND RITONAVIR IN ITS PURE AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

UV Derivative Spectrophotometric methods for Method I Simultaneous determination Method II First derivative method of Atazanavir sulphate and Ritonavir in tablet were developed in the present work. Spectrophotometric method for Simultaneous and First order derivative method of Atazanavir sulphate and Ritonavir at λmax 249 nm and 239 nm respectively. The various parameters were studied according to ICH. The linearity lies between 5–30 µg/ml for Atazanavir sulphate for two methods and 15–90 µg/ml for Ritonavir for method I and 10-90 µg/ml for first order method. Method II First order derivative was measured at 254 nm and 264 nm being Zero crossing point for Atazanavir sulphate and Ritonavir respectively in methanol. The proposed method has estimated for Method I Atazanavir sulphate and Ritonavir 100.01±0.117, 100.86±0.123 and for method II Atazanavir sulphate and Ritonavir 100.29±0.117, 99.09±0.121 in tablet. All methods showed good reproducibility and recovery with % RSD less than 1

KEYWORDS: Atazanavir Sulphate (ATV), Ritonavir (RTV), Simultaneous Method and Validation

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INTRODUCTION

Ritonavir (RTV) [1] is an antiretroviral drug. It is from the class of protease inhibitor used to treat HIV infection and AIDS. Ritonavir is frequently prescribed with Highly Active Anti-Retroviral Therapy, not for its antiretroviral action, but as it inhibits the same host enzyme that metabolizes other protease inhibitors.[2-3] It has the structural formula which was presented in Figure 1. The chemical name of Ritonavir is (5S, 8S, 10S, 11S)-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis (phenyl methyl)-2,4,7,12-etraazatridecan-13-oic acid 5-thiazolyl methyl ester, having a molecular formula ofC37H48N6O5S2with a molecular weight 720.946 g/mol. Protease inhibitors, such as Ritonavir prevent viral replication by inhibiting the activity of proteases, e.g. HIV- 1 protease, enzymes used by the viruses to cleave nascent proteins for final assembly of new virions[4-9]. It is official in Indian Pharmacopoeia [10] and United States Pharmacopoeia [11].

Atazanavir sulphate (ATV) is introduced as azapeptide inhibitor of HIV-1 Protease. It is formulated as 1:1 sulphate salt. It was approved by USFDA on June 20, 2003. Literature survey revealed that Atazanavir was quantitatively assayed in biological fluids either individually [12] or in presence of other retroviral drugs using liquid chromatography [13-15]. Chemically , Atazanavir Sulphate is (3S , 8S, 9S ,12S) - 3, 12 – Bis (1,1-dimethylethyl) – 8 – hydroxyl -4 , 11 – dioxo – 9 - (phenyl methyl) – 6 - [[4-(2 – pyridinyl) phenyl] methyl] – 2 , 5 , 6 , 10 , 13 penta azatetrade anedioic acid dimethyl ester;1 - [4 - (pyridine – 2 – y l) phenyl] - 5S , 2 , 5-bis [[N (methoxy

carbonyl) – L – tert – leucinyl] amino] - 4S hydroxyl – 6 –phenyl – 2 – azahexane [16], with a molecular formula C38H52N6O7.H2SO4 and a molecular weight of 802.93[17]. The structure of Atazanavir is shown in figure 2. An extensive literature review on the methods reported for the simultaneous estimation of Atazanavir and Ritonavir gives out information that there are few separate methods reported for the quantitative estimation of Atazanavir sulphate in bulk, pharmaceutical dosage forms and in plasma by HPLC, likewise a very few methods have been reported for the quantitative estimation of Ritonavir by HPLC but till date no method has been reported for the simultaneous quantitative estimation of Atazanavir and Ritonavir by UV Spectrophotometry.[18-22]

The aim of this work is to develop and validate a simple, accurate and low cost analytical method by using UV spectrophotometry for the estimation of Atazanavir sulphate in bulk and pharmaceutical dosage forms.

MATERIALS AND METHOD

Apparatus

Jasco V-630 double beam spectrophotometer with 1 cm path length supported by Jasco Spectra manager software was used for spectral measurements. Shimadzu balance (AUX 220) was used for all weighing.

Reagents and Chemicals

Pharmaceutical grade Atazanavir sulphate and Ritonavir were supplied by Cipla Ltd., Mumbai, India. The Methanol was purchased from Merck Chemicals and commercially available tablets SYNTHIVAN (equivalent to 300mg of Atazanavir sulphate and 100mg of Ritonavir) one of Cipla Ltd. was purchased from market for analysis.

Preparation of Stock Solution

Equivalent weight of 10 mg of Atazanavir and equivalent weight of 10 mg of Ritonavir sample were accurately weighed and transferred into a 100 ml clean dry volumetric flask separately and about 25 ml of methanol was added and sonicated to dissolve it completely and volume is made up to the mark with the same solvent for both drugs. The final solution was of concentration 100µg/ml of both drugs (Stock solution). Aliquots were further prepared.

Determination of λ Max

From the above working standard solution, 1 ml was pipette out into a 10 ml volumetric flask for both drugs and the volume was made up to the mark with methanol to prepare a concentration of 10 µg/ml for both drugs. Then the sample was scanned in UV-VIS Spectrophotometer in the range 200-400nm using methanol as blank and the wavelength corresponding to maximum absorbance (λ max) was found to be 249nm for Atazanavir and 239nm for Ritonavir.

Construction of Calibration Curve for Atazanavir Sulphate and Ritonavir

Standard dilutions of each drug were prepared separately having concentrations of 5-30 µg/ml for ATV and 15-90 µg/ml for RTV. The absorbance of these standard solutions were measured at 249 nm and 239 nm for Simultaneous method and 254 nm and 264 nm for First order derivative method for ATV and RTV respectively and calibration curves were plotted.

Method 1: Simultaneous Equation Method: [23]

Method was based on simultaneous equation method of Vierodt. The method is applicable in the case of sample containing two drugs, each of which absorbs at the λ max of the other. Three equations were developed using absorptive

coefficient values as an X component. The content in the mixture was determined by using the following three component equations/ Carmer's rule:

$$\mathbf{Cx} = \frac{A_1\, a_{y2} - A_2\, a_{y1}}{a_{x1}\, a_{y2} - a_{x2}\, a_{y1}} \quad \text{..... Eq. (i)}$$

$$\mathbf{Cy} = \frac{A_1\, a_{x2} - A_2\, a_{x1}}{a_{x1}\, a_{y2} - a_{x2}\, a_{y1}} \quad \text{.....Eq. (ii)}$$

Where,

C_x and C_y are the concentrations of ATV and RTV respectively. a_{x1} and a_{x2} are absoptivities of ATV at λ_1 and λ_2. a_{y1} and a_{y2} are absoptivities of RTV at λ_1 and λ_2. From the absorbance value obtained of all the two λ max i.e.249 nm for ATV and 239 nm for RTV, absorptive were calculated and shown in table 1.

Method 2: First Order Derivative Spectroscopy [23]

The first order derivative wavelengths consider for ATV 264 nm and for RTV 254 nm,

Assay of Atazanavir Sulphate and Ritonavir Tablet (Synthivan)

10 mg of Atazanavir and 10 mg of Ritonavir working standard were accurately weighed and transferred into a 100 ml clean dry volumetric flask and about 25 ml of methanol was added and sonicated to dissolve it completely and volume is made up to the mark with the same solvent (Stock solution). Further from the above stock solution 3ml of Atazanavir & 1ml of Ritonavir is pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent. Results are shown in table 2.

Method Validation: [24]

The method validation parameters like linearity, precision, accuracy, repeatability, limit of detection and limit of quantitation were checked as per ICH guidelines.

Linearity and Range

The linearity for ATV and RTV were determined at some concentration levels. For ATV and RTV from 5-30 µg/ml and 15-90 µg/ml using working standards.

Precision

The precision of the method was evaluated by interday and intraday variation studies. In intraday studies, working solutions of standard and sample were analyses thrice in a day and percentage relative standard deviation (% RSD) was calculated. In the interday variation studies, working solution of standard and sample were analysed on three consecutive days and percentage relative standard deviation (% RSD) was calculated. The data is shown in table 4-5.

Accuracy

The accuracy of the method was determined by recovery studies. The recovery studies were performed by the

standard addition method at 80%, 100% and 120% level and the percentage recoveries were calculated and are shown in Table 4-5.

Limit of Detection and Limit of Quantitation

The Limit of Detection (LOD) is the smallest concentration of the analyte that give the measurable response. LOD was calculated using the following formula and shown in Table 4 5.

LOD = 3.3 (σ / S)

Where, S = slope of calibration curve, σ = standard deviation of the response.

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in Table 4-5.

LOQ = 10 (σ / S)

Where, S = slope of calibration curve, σ = standard deviation of the response.

RESULST AND DISCUSSIONS

In present work, new method, namely, simultaneous equation method was used for the simultaneous spectroscopic estimation of ATV and RTV in commercially available dosage form. The concentration in range of 5-50µg/ml for ATV and 15-90µg/ml of RTV and two sampling wavelengths 249 nm and 239 nm for simultaneous estimation method and 264 and 254 nm for first order method for ATV and RTV respectively. The calibration curves constructed in the range of expected concentration 5-50 µg/ml for ATV and 15-90 µg/ml for RTV). The representative equation analysis was Y=0.0299x+0.0047, Y=0.02x - 0.0165 method I and II for ATV with regression coefficient 0.9988, 0.9994 respectively and Y=0.0128x-0.071, Y= 0.0112x - 0.0158 method I and II for RTV for RTV with regression coefficient 0.9996, 0.9978 respectively (Table 5, 6) LOD were found to be 0.0853, 0.2069 method I and II for ATV and 0.0831, 0.3880 method I and II for RTV respectively. LOQ were found to be 0.2519, 0.627 method I and II for ATV and 0.2519, 1.1758 method I and II for RTV respectively. The ATV and RTV in the sample indicated a satisfactory intra-day variability and inter-day variability (SD of ATV 0.011, 0.1058 and 0.023, 0.049 for method I and II, SD of RTV 0.0108, 0.116 and 0.0211, 0.114 for method I and II). A good accuracy of the method was verified with mean recovery of 100.01±0.117, 100.29±0.117 method I and II for ATV and 100.86±0.123, 99.09±0.121 method I and II for RTV (Table 4-5).

CONCLUSIONS

UV spectrophotometric methods for ATV and RTV were developed separately in bulk and tablet dosage form by, Simultaneous equation method. Further, UV Spectrophotometric methods for the simultaneous estimation by First order analysis of ATV and RTV were in bulk and combined dosage form. The methods were validated as per ICH guidelines. The standard deviation and % RSD calculated for these methods are <2, indicating high degree of precision of the methods. The results of the recovery studies showed the high degree of accuracy of these methods. In conclusion, the developed methods are accurate, precise and selective and can be employed successfully for the estimation of ATV and RTV in bulk and pharmaceutical dosage form.

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APPENDIX

Table 1: The Absorptivity Values of ATV and RTV in Proposed Method I and Method II

Absorptivity Values	249 Nm	239 Nm	264 Nm	254 Nm
Ax_1	0.02719	-	0.1510	-
Ax_2	-	0.01984	-	0.1875
Ay_1	0.00685	-	0.1246	-
Ay_2	-	0.01148	-	0.0851

Table 2: Table Shows Result of Analysis Data

Parameter	Method I		Method II	
	ATV	RTV	ATV	RTV
Drug content	100.14	99.44	101.17	99.75
S.D	0.372	0.112	0.523	0.252
%RSD	0.370	0.111	0.515	0.251

Table 3: Table Shows Result of Recovery Study

Drug	Amount Taken Mg	Amount Added		% Recovery	
		%	Mg	Method I	Method II
ATV	240	80%	240.1	100.02	100.08
RTV	80	80%	80.53	100.32	100.09
ATV	300	100%	300.2	100.03	100.04
RTIO	100	100%	98.9	99.94	100.00
ATV	360	120%	358.9	99.94	99.90
RTV	120	120%	121.1	100.08	102.1

Table 4: Table Shows Result of Validation Parameter for Simultaneous Equation Method

Sr. No	Parameters	Result of Method I	
		ATV	RTV
1	Absorption (nm)	249	239
2	Linearity range (µg/ml)	5-30µg/ml	15-90 µg/ml
3	Standard regression equation	Y= 0.0306x - 0.0038	Y=0.0129x-0.0133
4	Correlation coefficient (r2)	0.9988	0.9996
5	Accuracy (% recovery± SD)	100.01±0.117	100.86±0.123
6	Precision (% CV) Intraday Interday	 100.06 100.19	 100.11 100.09
7	LOD	0.0853	0.08314
8	LOQ	0.258	0.25198

Table 5: Table Shows Result of Validation Parameter for First Order

Sr. No	Parameters	Result Of Method II	
		ATV	RTV
1	Absorption (nm)	264	254
2	Linearity range (µg/ml)	5-30µg/ml	10-90 µg/ml
3	Standard regression equation	Y=0.02x - 0.0165	Y=0.0112x - 0.0158
4	Correlation coefficient (r2)	0.9994	0.9978
5	Accuracy (% recovery± SD)	100.29±0.117	99.09±0.121
6	Precision Intraday Interday	 101.03 100.29	 100.1 100.01
7	LOD	0.20691	0.3880
8	LOQ	0.627	1.1758

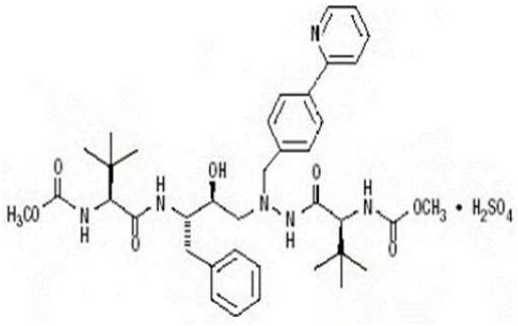

Figure 1: Ritonavir

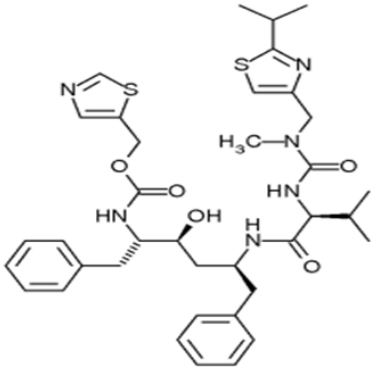

Figure 2: Atazanavir Sulphate

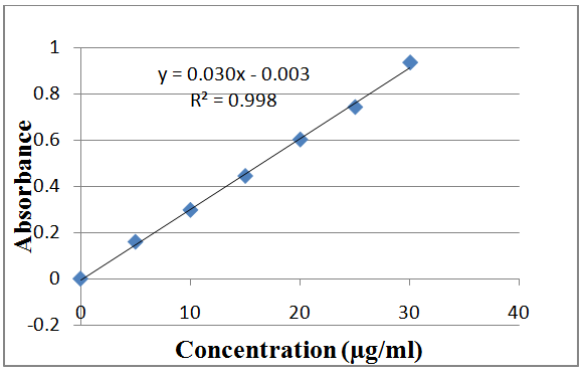

Figure 3: Calibration Curve of ATV

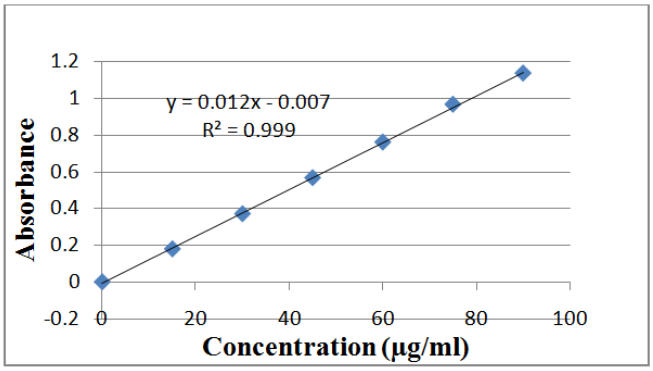

Figure 4: Calibration Curve of RTV

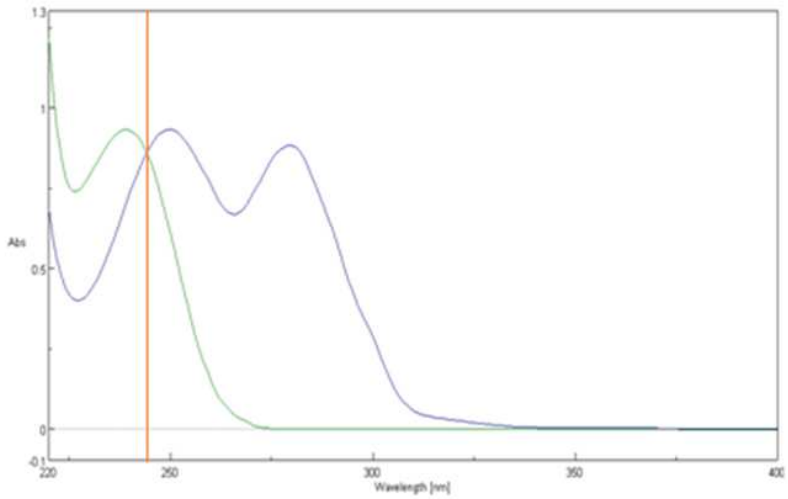

Figure 5: Overlain Spectra of ATV and RTV

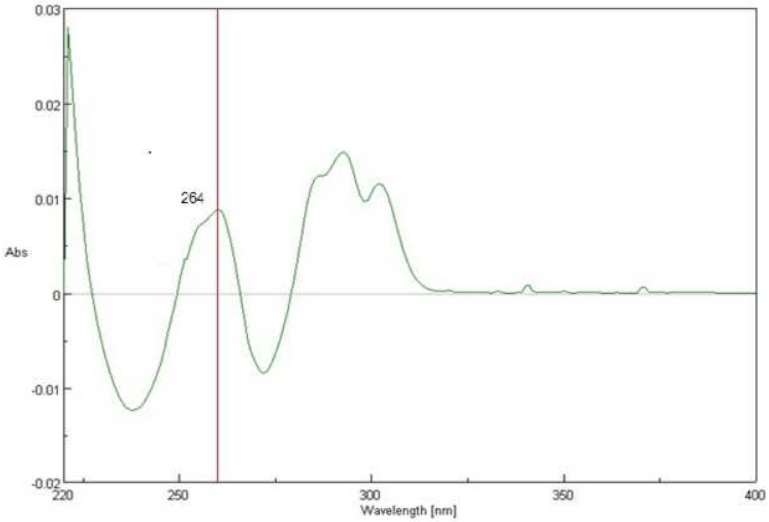

Figure 6: First Order Spectra of Atazanavir Sulphate

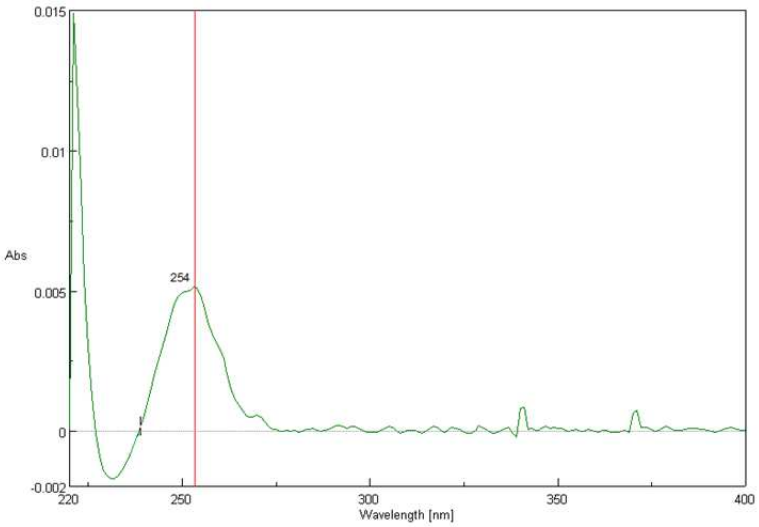

Figure 7: First Order Spectra of Ritonavir